EFFICACY OF BEEF CARCASS SURFACE TRIMMING TO REDUCE OR ELIMINATE *Escherichia coli* O157:H7 SURROGATES FROM SUBSEQUENT SUBPRIMALS

A Thesis

by

BRITTANY ANISE LASTER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Animal Science

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Approved by:

Co-Chairs of Committee,	Jeffrey W. Savell
	Kerri B. Harris
Committee Member,	Alejandro Castillo
Head of Department,	H. Russell Cross

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ABSTRACT

Efficacy of Beef Carcass Surface Trimming to Reduce or Eliminate *Escherichia coli* O157:H7 Surrogates from Subsequent Subprimals. (December 2010)

Brittany Anise Laster, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Jeffrey W. Savell
Dr. Kerri B. Harris

This study was conducted to determine the effectiveness of trimming the original external carcass surfaces from subprimals during fabrication on the reduction of surrogates for *Escherichia coli* O157:H7. Carcass sides from five cattle (n = 10 sides) were inoculated along the pattern hide opening before entering the blast chill cooler with a gelatin slurry containing a bacterial cocktail of three rifampicin-resistant, nonpathogenic *E. coli* Biotype I strains. Following a 48 h chill, sides were fabricated to produce eight subprimals (brisket, chuck, clod, rib, bottom round, top sirloin, short loin, and inside round). Microbiological samples were taken from the original carcass fat surface area, initial lean surface area, trimmed fat surface area (where applicable), and trimmed lean surface area (where applicable). Trimming of the external fat surfaces reduced ($P < 0.05$) microbiological counts on the newly exposed lean surfaces of all eight subprimals during fabrication. However, these data also indicated that fat and lean surfaces that were not initially exposed to contamination became contaminated during the fabrication process. Trimming external surfaces reduces levels of pathogens, but

under normal fabrication processes, pathogens may still be spread to the newly exposed surfaces.

DEDICATION

I dedicate this work to my family and close friends. Without the love and support of family and friends, none of my achievements would be possible.

ACKNOWLEDGEMENTS

This project was funded partially by The Beef Checkoff as part of their ongoing mission to increase beef safety**.** I would like to say thanks to my friends, colleagues, and the faculty and staff for making my time at Texas A&M University a great experience.

I thank my committee co-chairs, Dr. Savell and Dr. Harris, for allowing me the opportunity to further my education in meat science and food safety. Your guidance and support throughout my graduate career is greatly appreciated. My appreciation also goes to my committee member, Dr. Castillo, for your guidance and support throughout the course of this research. I also thank Lisa Lucia for all of her assistance.

Completing this study would have not been successful without the hard work, dedication, and support of my fellow graduate students: Ashley Haneklaus, Laura May, Will Wiederhold, Jacob Lemmons, Miles Guelker, and John Arnold. Thank you so much for helping me throughout this project and stepping in when I was sick. Thanks also to the following undergraduate student workers: Haley Grimes, Kayla Nelson, Trace Booth, and Julianne Riley.

Special thanks to Ray Riley and the staff at the Rosenthal Meat Science and Technology Center for allowing me to complete this work in their facility. I also thank the microbiology graduate students who took the time to help in the laboratory: Keila Perez, Mary Pia Cuervo, Mariana Villarreal Silva, Megha Adavi, and Holly Edwards.

Finally, thanks to my parents for their encouraging words. To my older sister, Candice, I love you so much, and I will keep looking to you for guidance and support. I am thankful to everyone that has impacted my life in some way.

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CHAPTER I

INTRODUCTION

During 2007 and 2008, the beef industry suffered from an increased number of positive *Escherichia coli* O157:H7 results, recalls, and related illnesses (*56, 57*). One area of current concern relates to *E. coli* O157:H7 contamination of beef products intended for the production of non-intact products. Non-intact products include beef that has been enhanced by vacuum tumbling, mechanically tenderized by cubing, needle injected to incorporate a marinade, or subjected to a comminution process such as grinding, chopping, or mincing (*55*). Pathogens may be introduced below the surface of these products as a result of these processes.

Many further processors utilize purchase specifications that require the application of a validated microbial intervention to support the decision that *E. coli* O157:H7 is not a reasonably likely to occur food safety hazard. In addition, survey data collected in 2004 by Kennedy et al. (*28*) did not detect *E. coli* O157:H7 on the surfaces of subprimals. However, the United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) continues to question the ability of further processors to support their decisions that *E. coli* O157:H7 is not a reasonably likely to occur food safety hazard on the raw materials used to produce non-intact products.

This thesis follows the style of *Journal of Food Protection*.

The beef industry has continued to search for ways to improve the safety of beef by reducing *E. coli* O157:H7 contamination. Studies have concluded that the primary sources of contamination during beef slaughter are fecal shedding and hides (*2, 16*). The efficacy of hide washes (*1, 5*), water washes (*7, 34*), hot water washes (*7, 8, 9, 19, 34*), steam pasteurization (*18*), and organic acid rinses (*8, 10, 11, 22*) to reduce pathogens has been reported.

Previous studies have evaluated the efficacy of trimming (*8, 22*) and trimming combined with other interventions (*8, 19*) in decontamination of carcasses. Results showed that the removal of visible fecal contamination by trimming alone reduced bacterial counts (*8, 22*), and when trimming was combined with treatments such as water wash, hot water wash, and organic acid sprays, reductions were also obtained (*8, 19*). However, no research has evaluated the ability to reduce bacterial levels by trimming exterior carcass surfaces during normal fabrication processes.

Therefore, three surrogate microorganisms were utilized to determine if trimming during fabrication was effective in reducing bacterial levels. Surrogate microorganisms are non-pathogenic microorganisms that grow, survive, and have resistant properties similar to specific pathogens. In addition, the potential for transferring contamination to the newly exposed surfaces was evaluated.

CHAPTER II

REVIEW OF LITERATURE

Foodborne illnesses occur in the United States every year due to cross contamination during harvest, fabrication, food handling, and in-home preparation by consumers. Research by Mead et al. (*37*) estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year. The most common pathogens causing illness include *Salmonella* Typhimurium, *E. coli* O157:H7, *Listeria monocytogenes, Clostridium perfringens,* and *Staphylococcus aureus.* Raw meat and poultry are common sources of foodborne illness due to the presence of pathogenic organisms (*50*).

Escherichia coli

E. coli was first discovered by bacteriologist Theodor Escherich in 1885. *E. coli* is a Gram-negative, mesophilic, non-sporing, facultative anaerobic microorganism that is commonly found in warm-blooded animals, including the human digestive tract (*25, 58*). The bacterial cell has a rod shape and flagella with a peritrichous arrangement when present. *E. coli* is commonly found in feces and water that has been exposed to feces (*3, 16, 17, 20, 21, 25, 58*). *E. coli* grows at temperatures ranging from 7-50°C with optimum growth at 37°C. Most strains of *E. coli* are harmless to healthy humans and are ubiquitous to nature (*25, 58*). However, there are a select few that cause infections in the central nervous system and gastrointestinal tract.

Pathogenic *Escherichia coli*

There are six virulence groups of *E. coli* that are recognized: enteroaggregative *E. coli* (EAggEC), diffusely adhering *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC) (*24, 25, 30, 31, 39, 43, 49*). These categories are based on virulence properties, interactions with the intestinal mucosa, clinical set of symptoms, epidemiology, and distinct O:H serotypes (*25, 30*). Serotypes were first distinguished by Kauffman (*27*) where he established the foundation for differentiating lipopolysaccharide somatic-O antigens, flagella-H antigens, and polysaccharide-K antigens.

EAggEC forms a mass similar to "stacked bricks" when they adhere to HEp-2 cells (*25*) and is differentiated from strains of EPEC by a particular pattern of adherence to Hep-2 cells that is clearly distinguishable from both localized and diffuse adherence (*30*). Some strains of EAggEC produce Shiga-like toxin know as enteroaggregative *E. coli* heat-stable enterotoxin-I (EAST-I)(*25, 30*). EAggEC strains play a major role in causing children to have persistent diarrhea that can last as long as 14 days (*24, 25*). Jalaluddin et al. (*24*) conducted a study to determine the involvement of toxin with the mechanisms of EAggEC diarrhea. Results from this study showed that none of the strains produced toxin although some strains can produce EAST-I (*24*).

ETEC strains attach and colonize in the small intestines by means of fimbrial colonization where they produce one or two enterotoxins (*25, 30, 31, 39, 43*). Diarrhea is

induced with the presence of these enterotoxins in the small intestine. The symptoms of ETEC infection include watery diarrhea, nausea, abdominal cramps, and low-grade fever. ETEC infection is a major cause of infant diarrhea and is a common incident in developing countries where the water supply and sanitation is limited. The incidence of ETEC in developing countries is high in infants and children under 2 years of age (*43*). ETEC infection is a major cause of traveler's diarrhea, and people traveling to developing countries have a higher chance of being infected with ETEC (*25, 30, 39, 43*).

The EIEC group is formed by strains capable of causing invasive dysenteric diarrhea. These strains are different in serotype to ETEC and EPEC, and more similar to *Shigella* (*30*). As with *Shigella*, EIEC have the ability to invade epithelial cells and take over with intracellular multiplication, which leads to invading neighboring epithelial cells. EIEC often resemble *Shigella* in being non-motile and unable to ferment lactose. Symptoms of EIEC infection include fever, severe abdominal cramps, lethargy, blood poisoning, and watery diarrhea followed by dysentery consisting of bloody stools with mucus. Foods are the main vehicle for transmission; however, person-to-person transmission is a possibility as well (*25, 30, 39, 51*).

EPEC strains do not develop the heat-labile and heat-stable enterotoxins found in ETEC, or do they exhibit the invasiveness of EIEC. They exhibit distinct patterns of microcolonies on epithelial cells called localized adherence (*25, 30, 39*). After local adherence takes place, attachment and effacement lesions are produced and involve microvilli destruction (*30*). This virulence factor is the most important in distinguishing EPEC strains. EPEC infection causes fever, lethargy, vomiting, and diarrhea with large

amounts of mucus with little blood present (*30, 39*). EPEC illness is more severe in infants under 1 year of age (*25*).

EHECs were first recognized as human pathogens following two multistate outbreaks of hemorrhagic colitis (HC) (*25, 30, 31, 38, 39*). The EHEC group is similar to EPEC in the way they posses common genes, and attachment-effacement lesion production. The traits that differentiate EHEC from EPEC are that EHEC strains invade the large intestines only and produce large amounts of Shiga-like toxins (verotoxin) (*25, 38*). There are at least 130 EHEC serotypes that have been recovered from human patients that cause hemorrhagic colitis. Two major non-O157 EHEC serogroups that have been associated with cases of hemorrhagic colitis are O26 and O111 (*38*). *E. coli* O157:H7 is considered the prototype of this group and the predominant cause of EHEC-associated illness in the United States (*25, 38*).

***Escherichia coli* O157:H7**

E. coli O157:H7 was first recognized as a foodborne pathogen following two outbreaks of hemorrhagic colitis in the United States that was linked to the consumption of hamburgers (*45, 59*). It was not until a large multistate outbreak in 1993 that took the lives of four children that reports of *E. coli* O157:H7 outbreaks gained publicity at an increasing rate (*4, 44*). *E. coli* O157:H7 organisms attach to the mucosal surfaces of the large intestines and cause attaching-effacing lesions at the site of attachment. High levels of Shiga-like toxins are released to the cell surfaces causing damage to the large intestine resulting in bloody diarrhea (*4, 38*). Children, the elderly, and those with a weakend immune system are the most susceptible to infection due to their level of immunity being

low. Clinical symptoms include watery diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and death (*4, 25, 30*). Patients with *E. coli* O157:H7 infection express different symptoms depending on the level of pathogen present and their immunity levels, which plays a role in their susceptibility to the infection. Following the incubation period in the large intestine for 3-4 days, watery diarrhea usually occurs. In 25-50% of cases, symptoms are mild and resolve without progression (*4*); if the disease progresses, bloody diarrhea begins with blood ranging from small amounts to stools that are entirely blood (*4, 38*).

The most common mode of transmission of *E. coli* O157:H7 is the ingestion food or water that has been contaminated. The majority of *E. coli* O157 infection outbreaks are traced back to beef (*4, 25, 39, 44*) with ground beef as the primary cause of outbreaks in the United States (*4*). The largest outbreak in 1993 dealt with the consumption of undercooked hamburgers from a fast-food chain (*4, 44*). Other foods that have been associated with outbreaks of *E. coli* O157:H7 are dry-cured salami, roast beef, unpasteurized apple juice and cider, raw milk, cheese and butter, spinach, lettuce, coleslaw, and sprouts (*3, 25, 44*).

Other modes of transmission include person-to-person direct contamination, and direct contamination from an animal (*4, 15, 25, 39*). Contamination between people commonly happens in settings such as nursing homes and day care facilities (*4*). A study by Hancock et al. (*21*) focused on the prevalence of *E. coli* O157 in feedlot cattle in 13 states in the United States. Results from this study showed that 1.6% of fecal samples collected tested positive for *E. coli* O157:H7 while 2.4% of fecal samples tested positive

for *E. coli* O157. From the samples collected, *E. coli* O157:H7 was isolated from 61.0% of feedlots and *E. coli* O157 was isolated from 63.0% of feedlots (*21*). A later study by Sargeant et al. (*46*) also observed the prevalence of *E. coli* O157:H7 in feedlot cattle and found higher prevalence of *E. coli* O157:H7 than Hancock et al. (*21*) previously conducted. However, results from Hancock et al. (*20*) concluded three theories: first, *E. coli* O157 may have multiple species that can act as reservoirs, which may or may not include cattle; second, *E. coli* O157 may be able to transiently colonize in many species, but one or more species also can serve as the reservoir; and third, *E. coli* O157 could have an environmental reservoir, such as the sedimentary layer of water-troughs.

Because many outbreaks of *E. coli* O157:H7 have been linked to beef more than any other food source, cattle have been typically considered the primary reservoir in the United States (*13, 17*). Elder et al. (*16*) conducted a study looking at the frequency of *E. coli* O157:H7 in feces and on hides from beef cattle prior to slaughter, how many carcasses were being contaminated during processing, and whether there was a relationship between the two. Results showed that *E. coli* O157:H7 was transferred to carcasses during slaughter through fecal contamination. Cattle hides have been implicated as important sources of carcass contamination during slaughter (*16, 42*). Elder et al. (*16*) found that 72% of lots had at least one fecal sample positive for *E. coli* O157, and 38% of the same lots had at least one hide sample positive for this pathogen.

Pre-harvest interventions

At feedlots, muddy pens and crowding of cattle may possibly increase contamination on cattle and carcasses (*32, 47*), but there are not enough data to support this theory. However, USDA and the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has recognized receiving and holding of cattle at plants as major sites of contamination during harvest (*34*). Elder et al. (*16*) evaluated the frequency of *E. coli* O157:H7 in feces and on hides of cattle prior to harvest and the frequency of carcass contamination during harvest. They reported that the prevalence of carcass contamination was higher than that of fecal and hide prevalence. Some cattle presented for harvest tested negative for *E. coli* O157:H7; however, carcass samples from these same cattle tested positive for *E. coli* O157:H7 suggesting that cross-contamination may be occurring during harvest from direct contact with workers, knives, equipment in the slaughter facility, carcass-to-carcass contamination, or indirect contamination from water and air (*16*). McEboy et al. (*36*) evaluated the occurance and distribution of *E. coli* O157:H7 on beef carcasses and the prevalence of *E. coli* O157:H7 in fecal and rumen samples. Results showed that *E. coli* O157:H7 can be present in the feces and rumen of cattle at slaughter and cross-contaminate carcasses during hide removal and the bunging process (tying off the anus). With these possibilities of cross contamination, interventions have been put in place before and after harvest.

Loneragan and Brashears (*32*) conducted a review of pre-harvest interventions for harvest-ready feedlot cattle. Two options were discussed concerning pre harvest interventions: modifying management practices that are currently implemented, and

developing new management practices that will improve areas that need improvement. Pre-harvest interventions include vaccines, direct-fed microbials, bacteriophage, manure and cattle pen surface treatments (*3, 32*). The most popular direct-fed used currently in the beef industry is a *Lactobacillus*-based direct-fed microbial (*32*). In a study by Younts-Dahl et al. (*60*), high levels of *Lactobacillus acidophilus* (HNP51) in feed helped reduce the likely occurrence of cattle shedding *E. coli* O157:H7 compared to cattle not fed this probiotic. Another probiotic that can possibly benefit in reducing shedding of *E. coli* O157:H7 is *Enterococcus*. Data are limited for this probiotic, and further research is needed to validate its use in cattle (*32*). Vaccines are already used in the beef industry to reduce the incidence of disease in cattle. A vaccine to reduce *E. coli* O157:H7 would be given to cattle to improve public health and have little benefit for the cattle (*32*). Sodium chlorate is another option to use to reduce *E. coli* O157:H7. Sodium chlorate is added to feed and drinking water for cattle, and when ingested it reduces populations of *E. coli* O157:H7 in the feces and in the intestinal content of cattle (*32*).

Post-harvest interventions

Current interventions used during harvest to help in the reduction of hazards include trimming (*7, 8, 22, 34*), water washing (*7, 8, 19, 22, 34*), hot water rinsing (*7, 8, 9, 19, 34*), steam vacuuming (*7, 14, 18, 34*), steam pasteurizing (*7, 18, 34*), and organic acid spraying (*7, 8, 10, 11, 22, 34*). Other interventions that have been reported on reducing carcass contamination are chemical dehairing, low-voltage pulsed electricity, ozonated water, and hydrogen peroxide (*7*).

Hardin et al. (*22*) evaluated water washing by hand followed by washing in an automated spray cabinet, the water washing treatments followed by a 2% lactic acid spray, and the water washing treatments followed by a 2% acetic acid. Results showed that a carcass wash followed by a 2% acid spray was more effective then water wash alone in reducing pathogens, and lactic acid produced higher reduction of bacterial levels than acetic acid. Studies have shown that lactic or acetic acid sprays, when applied at 55°C, can achieve reduction of *E. coli* O157:H7 levels even though *E. coli* O157:H7 has been reported to be resistant to low pH environments (*8, 22*). Castillo et al. (*8*) reported that the highest reductions were obtained by a treatment of water wash followed by a combined treatment of hot water (95°C) and 2% lactic acid spray. Hot water treatments are different from regular water washes in that the water reaches temperatures higher than 74°C and may be used as a sanitizing intervention (*7*). Hot water treatments (77°C) combined with cold-water spray-washing achieved reductions in visible contamination and coliform counts (*19*). Castillo et al. (*9*) compared the application of immediate and delayed (30 min) treatments of warm carcass wash and warm carcass wash followed by a hot water treatment on the reduction of pathogens. All treatments showed reduction of initial counts of *E. coli* O157:H7 of 3.7 log, and coliform count reductions of 3.3 log. There was no difference on the effect of application time.

The application of steam during slaughter can be used to reduce bacterial counts (*14, 18*). Steam pasteurization has the ability to achieve great reductions in bacterial counts, however, the equipment is expensive and the addition of the treatment to the harvest process may not be necessary when other interventions such as hot water or

lactic acid treatments achieve the same, if not greater, reductions in bacterial counts (*7*). There are two methods of applying steam to carcasses. The first method is exposing beef sides to an atmospheric chamber of steam that raises the meat surface to pasteurizing temperatures quickly. The second method, steam vacuuming, is the method of delivering hot water and/or steam through a hand-held vacuum head fitted with nozzles to selected areas where visual contamination is common on carcass surfaces (*18*). Steam vacuuming was designed as a spot-cleaning treatment to clean and sanitize small areas of contamination while removing physical contaminants such as fecal, milk, and ingesta through a vacuum (*7, 53*). In a review on different methods of reducing microbial contaminants on beef carcass, Castillo et al. (*7*) described an in-plant study of two steam-vacuuming units, indicating that steam vacuuming reduced microbiological contamination and improved visual appearance of carcasses for which knife trimming would have been required by the inspector.

FSIS requires zero tolerance for visible fecal material as a food safety standard (*54*). To achieve this standard, harvest facilities handle carcasses and carcass parts to prevent contamination with fecal material and remove contamination if it occurs. Hardin et al. (*22*) reported that trimming was useful for removing visible fecal contamination from carcass surface regions, but was not as effective in reducing bacterial counts as was a wash/acid treatment. Trimming alone should not be used as the primary method of decontaminating carcasses because a visually clean carcass does not mean microorganisms are not present on carcasses at unsafe levels. Instead, trimming should be followed by a subsequent step of decontamination such as water washing, hot water

spraying, steam vacuuming, or an organic acid spray (*7, 19, 22, 34*). In order for these interventions to remain effective, equipment should be properly cleaned and sanitized and employees must be trained and updated on new strategies, standard operating procedures (SOPs), good manufacturing practices (GMPs), and sanitation standard operating procedures (SSOPs).

Fabrication interventions

The majority of studies that discuss trimming as an intervention or part of an intervention are using the method during harvesting procedures (*7, 19, 22, 29, 34*). Heller et al. (*23*) conducted a study evaluating methods of decontaminating beef subprimals that were intended for blade tenderization and moisture enhancement. These methods included surface trimming using good manufacturing practices (removal of surface fat with a sterile knife in approximately one single swipe), hot water, warm 2.5% and 5% lactic acid, and 2% activated lactoferrin (AL) followed by warm 5.0% lactic acid. Results from this study showed that *E. coli* O157:H7 had a survival rate of 70.2% after trimming, 72.2% after both hot water and warm 2.5% lactic acid, 68.5% after warm 5% lactic acid, and 74.2% after AL followed by warm 5% lactic acid. This study concluded that applying antimicrobial interventions before mechanical tenderization reduced the levels of contamination to non detectable levels (*23*). As mentioned earlier, trimming subprimals during fabrication has been theorized to reduce levels of *E. coli* O157:H7, however, this has not been supported by peer-reviewed research.

Surrogates

Surrogates are organisms that are used specifically as biological indicators that can mimic the behavior of one or more pathogens. These surrogates can be used inside a plant without intentionally introducing pathogens to food processing facility. Marshall et al. (*35*) compared five indicators with five isolates of *E. coli* O157:H7. The isolates were challenged to seven different antimicrobial treatments and the results showed that these indicators in a combined cocktail served to evaluate and validate antimicrobial intervention for beef carcasses. Niebuhr et al. (*40*) used these *E. coli* biotype I isolates to compare the responses of these surrogates to a mixed culture of *Salmonella.* Results from this study showed that four of the five surrogates used had a higher survival rate than the *Salmonella* culture when exposed at the same antimicrobial interventions.

The U.S. Food and Drug Administration (FDA), defines the supreme surrogate as being the pathogen itself and having its pathogenic abilities removed by genetic engineering modifications (*52*). However, the presence of these modified pathogens in harvest facilities can lead to false positives during routine testing. So a better approach to defining the ideal surrogate includes the following characteristics: nonpathogenic, with thermal or chemical inactivation equal numerically and kinetically to the target pathogen, and durability in foods equal to the target organism such as pH stability, refrigeration stability, and atmosphere tolerance. The surrogate must be easily identifiable, isolated, and enumerable under rapid and inexpensive detection systems, easily differentiable from natural occurring bacteria, and very stable so results can be repeatable (*52*).

CHAPTER III

MATERIALS AND METHODS

Bacterial cultures

This project was originally designed to utilize three nonpathogenic protein-marked *E. coli* Biotype I strains that were identified through previous studies (*35, 40*) and were transformed in the Food Microbiology Laboratory at Texas A&M University to produce green, red, or yellow fluorescing proteins and to express ampicillin-resistance properties (100 µg/liter). These isolates have been deposited with the American Type Culture Collection under accession numbers BAA-1427, BAA-1428, and BAA-1430. These marker organisms were designed to be utilized in a "cocktail" to represent possible contamination with enteric pathogens of fecal origin such as *Salmonella* or *E. coli* O157:H7. Through previous research, these marker organisms demonstrated identical thermal and acid resistance to *E. coli* O157:H7 (*6, 35, 40*). Based on preliminary trials in this current study, the red fluorescing protein-marked organism failed to consistently fluoresce and as a result, all three strains were replaced with *E. coli* Biotype I strains (BAA-1427, BAA-1428, and BAA-1430) that were transformed to express rifampicin resistance (100 µg/liter). The rif-resistant *E. coli* Biotype I strains were identified as *E. coli* #1, *E. coli* #3, and *E. coli* #14.

The three nonpathogenic *E. coli* Biotype I strains were obtained from the Food Microbiology Culture Collection (Texas A&M University, College Station, TX) and maintained at -80°C in cryocare vials (Key Scientific Products, Round Rock, TX). One

bead of each strain was transferred to tryptic soy broth (TSB; BD Diagnostics, Sparks, MD) and incubated (VWR incubator, Sheldon Manufacturing, Inc., Cornelius, OR) for 18±2 h at 35°C. One full loop of each cultivated strain was obtained using a sterile loop, transferred to TSB, and incubated for 18±2 h at 35°C. Procedures from Kaspar and Tamplin (*26*) were followed to develop rif-resistant strains. The rif-resistant strains *E. coli* Biotype I strains developed in this study were maintained on TSA slants as working stock cultures for propagations. Slants were incubated at 35°C for 18±2 h and kept at 25°C to be used within 30 days.

Because *E. coli* O157:H7 cannot ferment sorbitol (*12, 33*), MacConkey sorbitol agar (SMAC; MacConkey Sorbital Agar, Becton, Dickinson and Company, Franklin Lakes, NJ;) was used to determine if the strains were *E. coli* O157:H7. If results were positive for *E. coli* O157:H7, colonies would appear clear on SMAC. If negative for *E. coli* O157:H7, the colonies would not appear clear (*12*). As an additional preliminary test to determine if the rif-resistant strains were not *E. coli* O157:H7, colonies were taken from the SMAC and tested using the *E. coli* O157:H7 latex agglutination test kit (RIM *E. coli* O157:H7 Latex test kit, Remel, Lenexa, KS) to determine whether strains belong to the O157 serogroup. One colony was suspended into one drop of latex from the *E. coli* O157 test kit to see if it would agglutinate. Sheep blood agar (CDC Anaerobe 5% Sheep Blood Agar, Becton, Dickinson and Company, Franklin Lakes, NJ) was used as enrichment for the development of flagella. Colonies from each strain were taken from the sheep blood agar and tested using the *E. coli* O157:H7 latex agglutination test kit to determine whether strains belong to the H7 serogroup. One colony was suspended into

one drop of latex from the *E. coli* H7 test kit to see if it would agglutinate. Results were negative for both latex tests concluding that the rif-resistant microorganisms were not *E. coli* O157:H7.

These three rif-resistant strains were designed to be utilized in a "cocktail" to represent possible contamination with enteric pathogens of fecal origin such as *Salmonella* or *E. coli* O157:H7. Three trials were conducted and consisted of plating appropriate serial dilutions of the individual strains and the cocktail, which consisted of 3 ml from each strain mixed, on rif-TSA plates. Trials were conducted to test for consistency in growth and concentration of the cocktail as well as the individual strains. All plates were incubated at 35°C for 18±2 h. The concentration of the cocktail was approximately 9 $\log_{10}$ CFU/ml. Through previous research, these marker organisms have demonstrated identical thermal and acid resistance to *E. coli* O157:H7 (*6, 35, 40*). Growth curves were conducted and results indicated that there was no significant difference between the growth rates of the parent strains and the rif-resistant strains.

Preparation of gelatin inoculum

These microorganisms were inoculated in an opaque gelatin matrix that mimics fecal slurry. This type of procedure allows for inoculating carcasses without using actual feces, which would result in zero tolerance non-compliance in the harvesting facility. The gelatin mixture was prepared the day before inoculation to allow for the mixture to cool to room temperature (25°C).To prepare the gelatin mixture of 5.4 liters, 42 g of food-grade unflavored gelatin (Kraft Food North America, Tarrytown, NY) was placed

into 1 liter of boiling sterile 0.1% peptone water (PW; Peptone, Difco, Sparks, MD) and was allowed to dissolve for 5 min. This step was repeated to dissolve an additional 42 g of gelatin, and then both of the dissolved gelatins were combined with the remaining PW (3.4 liters) and poured into two 4 liter plastic beakers that were held for 18±2 h at room temperature (25°C).

Each rifampicin-resistant surrogate was cultured in 250 ml of TSB the day before inoculation and incubated at 35°C for 18±2 h. Following incubation, a bacterial cocktail was prepared by mixing equal volumes (200 ml) of each of the three cultures for a total of 600 ml, and the cocktail was used to inoculate the gelatin slurry. On each slaughter day, the gelatin slurry (5.4 liters) was poured into a polyethylene tank (2-gallon Ortho Heavy Duty Sprayer, The Fountain Group, Inc., New York Mills, NY) and capped. Immediately before spraying onto the carcasses, the surrogate cocktail (600 ml) was aseptically added to the sprayer containing the gelatin slurry. The tank was capped and shaken by hand for 15 s to distribute the cocktail throughout the gelatin slurry. The average concentration of the gelatin slurry following the addition of the cocktail was 7 $\log_{10}$ CFU/ml.

Harvesting and inoculation

A total of five head of cattle were harvested at the Rosenthal Meat Science and Technology Center (RMSTC) at Texas A&M University, College Station, TX, on two different dates. The beef slaughter process at this facility is an on-the-rail gravity flow procedure (*48*), and standard slaughter procedures were performed up to carcass

splitting. Following carcass splitting, samples were obtained from the external surface of the carcass to measure background flora before inoculation. Two 10-cm^2 samples were excised randomly using a sterile stainless steel borer, scalpel, and forceps and were composited (20-cm^2 total area) from three locations on the left side of each carcass. Sample locations included the round, rib/plate, and chuck areas. Before entering the blast chill cooler (-2 to 0°C), the normal pattern opening areas (e.g., brisket, plate, flank, round, and leg extremities) of both sides of each carcass were inoculated using the pump sprayer. The average flow rate of the gelatin inoculum was 23 s/carcass side for an average volume of 565 ml/carcass side. The flow of the slurry exiting the sprayer was calibrated before the first carcass inoculation for each harvest day. On each day of slaughter, 5 ml of the gelatin slurry was sampled in order to verify level and homogeneous distribution of the surrogates. Carcass sides remained in the blast chill cooler (-2 to 0°C) for approximately 48 h. The average initial count of the carcass following inoculation was 5 $\log_{10}$ CFU/cm^2.

Fabrication and microbiological testing

After chilling, each side was fabricated separately, and the forequarter and hindquarter were processed on different cutting tables. Fabrication followed general laboratory procedures outlined in Savell and Smith (*48*) to produce eight subprimals: rib, chuck, brisket, shoulder clod, inside round, outside round, short loin, and top sirloin. The beef band was separated from the chuck with a cut between the 5th and 6th ribs. The before trim (BT) rib fat samples were taken from the exterior carcass fat surface over the

M. longissimus thoracis. The BT lean samples for the rib were taken from the anterior exposed surface of the *M. longissimus thoracis* after the rib was separated from the chuck (6th rib interface). The rib was further processed in compliance with the Institutional Meat Purchase Specifications (IMPS) 112A (*41*) by removing all bones, lifter meat (*M. latissimus dorsi, M. rhomboideus, M. trapezius,* and *M. subscapularis*) with external surface fat, cartilage, and *ligamentum nuchae*. The after trim (AT) fat samples for the rib were taken on the intermuscular fat surface on the dorsal aspect of the ribeye exposed after the lifter meat was removed, and the AT lean samples were taken on the ventral lean surface exposed after the back ribs were removed.

The brisket was removed from the chuck by making a straight cut 2.5 cm from the end of the *M. pectoralis profundus* and at the cartilaginous juncture of the 1st rib and the sternum. The BT fat samples for the brisket were taken from the exterior carcass fat surface distal to the sternum. The BT lean samples were taken on the lean surface exposed after the sternum was removed because there were no other exposed lean areas that provided the appropriate sample size. The exterior carcass fat surface was trimmed to 0.6 cm. The AT fat samples for the brisket were taken on the newly exposed fat surface. The surface where the sternum was removed was trimmed flush with the lean, and the AT lean samples were taken on the newly exposed lean surface.

The shoulder clod was removed to include the *M. infraspinatus, M. triceps brachii,* and *M. teres major*. The BT fat samples were taken from the exterior carcass fat surface where the *M. cutaneus omobrachialis* was present. The BT lean samples were taken on the interior lean surface of the *M. infraspinatus, M. triceps brachii, and M.*

teres major muscles. Because the lean surface was the same before and after fabrication, AT lean samples were not taken. Following the trimming of the exterior carcass fat surface to 0.6 cm, the AT fat samples were taken from the newly exposed fat surface.

The BT fat samples for the chuck were taken on the external fat surface near the dorsal anterior portion of the chuck primal that is removed during production of the chuck roll. The BT lean samples for the chuck were taken on the *M. longissimus thoracis* face where the saw was used to separate the rib from the chuck (5th rib interface). The chuck was fabricated to comply with the specifications for the IMPS 116A (*41*) by removing all cartilage, *ligamentum nuchae*, lymph glands, and bones. On the chuck, the external fat was removed during fabrication, so AT fat samples were not taken. The AT lean samples for the chuck roll were taken on the dorsal aspect on the newly exposed lean surface where the external fat was removed.

The round was separated from the full loin between 4th and 5th sacral vertebrae and about 2.5 cm anterior to the knob of the aitch bone. The sirloin and short loin were separated between the 5th and 6th lumbar vertebrae and immediately anterior to the hip bone. The BT fat samples for the short loin were taken on the external fat surface over the *M. longissimus lumborum*. The BT lean samples for the short loin were taken from the *M. longissimus lumborum* face on the sirloin end where the top sirloin was separated from the short loin (6^{th} lumbar interface). The external fat surface was trimmed to 0.6 cm, and the AT fat samples were taken on this newly exposed surface. Because the short loin remained an IMPS 174 (*41*), the lean surface was not changed so an AT lean sample was not taken.

The sirloin was separated through the natural seam to obtain a top sirloin and bottom sirloin. The BT fat samples for the top sirloin were taken from the exterior carcass fat surface. The remaining portions of the ilium and sacral bones were removed. The BT lean samples for the top sirloin were taken on the ventral lean surface exposed after the bones were removed. The external fat surface of the top sirloin was trimmed to 0.6 cm, and the AT fat sample was taken on the newly exposed fat surface. The lean surface did not change after the bones were removed resulting in an AT lean sample not being taken.

The round was suspended on the rail with a j-hook and trolley for easier removal of the knuckle, inside round, and gooseneck. The inside round was separated from the bottom round and knuckle through the natural seam and was placed on the cutting table. The BT fat samples for the inside round were taken from the exterior carcass fat surfaces. The BT lean samples were taken on the ventral lean surface where the inside round was separated through the natural seam. Because the lean surface remained the same before and after fabrication, AT lean samples were not taken from the ventral surface; instead, the dried lean surface of the *M. semimembranosus* was trimmed, and the newly exposed lean surface was sampled. The exterior carcass surface of the inside round was trimmed to 0.6 cm, and the AT fat samples for the inside round were taken on the newly exposed fat surface.

For the gooseneck (IMPS 170) (*41*), the BT fat samples were taken from the exterior carcass fat surface. The BT lean samples were taken on the ventral lean surface of the *M. semitendinosus*, *M. gluteobiceps*, and *M. gastrocnemius*. The gooseneck was

processed further by removing the heavy connective tissue (epimysium), *popliteal* lymph gland, *sarcosciatic* ligament, *M. semitendinosus,* and *M. gastrocnemius* muscles to produce a bottom round flat (IMPS 171B) (*41*). The AT fat samples for the bottom round flat were taken on the newly exposed external fat surface. The AT lean samples for the bottom round flat were taken on the

lean surface exposed after the *M. semitendinosus, M. gastrocnemius*, heavy connective tissue (epimysium), *popliteal* lymph gland, and *sarcosciatic* ligament were removed.

During fabrication of the carcass sides, worker's knives, hooks, and steels were sanitized in a chemical sanitizer (Biquat, Birko Corporation, Henderson, CO) every 5 min. Following the fabrication of the first side, the plastic table tops were flipped before starting fabrication of the second side of the carcass to minimize contamination from one side to the next. The cutting lab was cleaned and sanitized between carcasses to minimize contamination between carcasses.

To ensure that cleaning and sanitizing practices removed any residual surrogate microorganisms, environmental samples were taken using sponges on randomly selected places on the slaughter floor, blast chill cooler, and research cutting lab. Before sampling, the sponge was moistened with 25 ml of sterile Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA), and the sample collection was achieved by firmly rubbing the damp sponge over a selected area of 400-cm^2 with a pre-moistened sterile sponge using the same procedure of 10 vertical and 10 horizontal passes.

All microbiological samples from subprimals were obtained by excising two 10-cm^2 x 2 mm thick samples using a sterile stainless steel borer, scalpel, and forceps, and compositing them (20-cm^2 total area). Each composite sample was placed into a sterile stomacher bag, placed in an insulated container containing refrigerants, and manually transported to the Food Microbiology Laboratory. Upon arrival, 99 ml of sterile 0.1% peptone was added to each stomacher bag. The sample then was pummeled for 1 min at 260 rpm using a Stomacher 400 (Tekmar Company, Cincinnati, OH). For each sample, counts of rifampicin-resistant *E. coli* surrogate microorganisms were determined by plating appropriate decimal dilutions on prepoured and dried rifampicin-tryptic soy agar (rif- TSA) plates. Rif-TSA was prepared by adding a solution of 0.1 g of rifampicin (Sigma-Aldrich Inc., St. Louis, MO) dissolved in 5 ml methanol (EM Science, Gibbstown, NJ) to 1 liter of autoclaved and cooled (55°C) TSA. The environmental samples were massaged for 1 min and plated on rif-TSA plates. The rif-TSA plates were incubated for 18±2 h at 35°C before counting and reporting the number of rif-resistant *E. coli* Biotype I per cm^2. Negative controls were taken before carcass inoculation to test for any rif-resistant contaminants.

Statistical analysis

Data were analyzed using PROC GLM of SAS (SAS Institute, Inc., Cary, NC). Least squares means were generated for main effects and separated using PDIFF option when appropriate with an alpha-level ($P < 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

This study used rifampicin-resistant surrogate microorganisms that would not typically be found in a commercial beef-processing establishment. To ensure that rif-resistant microorganisms were not naturally present on the carcasses, samples were obtained from non-inoculated sides as a control to determine the natural background flora. As expected, there were no detectable counts of rifampicin-resistant microorganisms on the non-inoculated sides (data not reported in tabular form).

After application of the inoculum and before chilling carcasses, samples were collected from three locations (round, plate, and brisket) along the pattern opening area to determine the initial level of rifampicin-resistant microorganisms on the carcass. There were no differences ($P > 0.05$) in counts from the inoculated carcasses when comparing slaughter days (Table 1), and there were no differences ($P > 0.05$) in counts for the brisket, plate, and round areas (Table 2) of the inoculated carcasses. These data support that the initial carcass counts were consistent between the two slaughter dates, and that the application covered all areas of the pattern opening. This consistent level of contamination is different than would be expected during a normal harvest process, but it was necessary to ensure proper application to determine the impact of trimming during fabrication.

After chilling, samples were taken during the fabrication process to determine the level of rifampicin-resistant microorganisms on both fat and lean tissues. The counts

from the exterior carcass fat surface of the brisket (Table 3) were higher ($P < 0.05$) compared to the counts from the newly exposed fat surface, initial lean surface, and as well as the newly exposed lean surface. These results show that the counts were highest ($P < 0.05$) on the fat surface from the exterior fat surface of the brisket and decreased ($P < 0.05$) as normal trimming of fat and removing of the sternum occurred.

For the chuck roll (Table 4), the counts of microorganisms for the exterior carcass fat surfaces were higher ($P < 0.05$) than the samples taken on the initial lean surfaces and the lean surfaces exposed during fabrication. There were no differences ($P > 0.05$) in counts of microorganisms between the initial lean surfaces and the newly exposed lean surfaces.

For the clod (Table 5), the counts of microorganisms on the exterior carcass fat surface were higher ($P < 0.05$) than the initial lean surface and the newly exposed fat surface. There were no differences ($P > 0.05$) in counts of microorganisms between the initial lean surfaces and the newly exposed fat surfaces. These results show that trimming the exterior carcass fat surface removed a significant amount of the inoculum.

The counts of microorganisms on the exterior carcass fat surface of the rib (Table 6) were higher ($P < 0.05$) when compared to the initial lean surface, the trimmed fat surface, and the trimmed lean surface. However, the counts on the newly exposed lean surface were higher ($P < 0.05$) than those of the initial lean surface, which indicates that additional contamination possibly occurred during fabrication.

For the bottom round (Table 7), there were no differences ($P > 0.05$) in log values of microorganisms of the exterior carcass fat surface and the newly exposed fat

surfaces compared to the initial and newly exposed lean surface samples. Due to the anatomical location of the bottom round and the fabrication process, these results are not surprising because fat surfaces could have potentially been exposed to the initial inoculum spray.

The counts on the exterior carcass fat surface of the short loin (Table 8) were higher ($P < 0.05$) than those of the trimmed external fat surface and the initial lean surface. There also were differences ($P < 0.05$) in counts of microorganisms for the top sirloin (Table 9) between the exterior carcass fat surface and both the initial lean surface and trimmed fat surface. These results demonstrate that as the exterior fat surface is removed, the interior fat and lean surfaces have lower levels of contamination. The results for the inside round (Table 10) show that the exterior fat counts were higher ($P < 0.05$) than the initial lean, as well as the trimmed fat and lean counts. There were no differences ($P > 0.05$) between the initial lean and the trimmed lean counts. However, trimmed fat counts were higher ($P < 0.05$) than both the initial lean and the trimmed lean counts. These results support that the contamination of the fat was reduced by trimming, and that the lean surfaces were possibly contaminated during fabrication.

Environmental samples were taken to ensure that the rifampicin-resistant microorganisms were removed from the facility. Samples were taken from several locations in the harvest floor area where the carcasses were inoculated, from the blast chill cooler where carcasses were chilled, and from the processing room where carcasses were fabricated. The counts of microorganisms from these areas were below detectable levels (not reported in tabular form).

Although there were variations in the microbiological counts from the impact of trimming exterior carcass surfaces during fabrication, this project showed that there was a general trend that counts from trimmed fat and trimmed lean surfaces were lower than initially exposed fat or lean surfaces. However, these data also indicated that fat and lean surfaces that were not initially exposed to contamination became contaminated during the fabrication process which indicates that trimming external surfaces results in reduced but not completely eliminated pathogens on the finished products.

CHAPTER V

CONCLUSION

The beef industry continues to be criticized by USDA-FSIS and consumer groups for not preventing illnesses associated with *E. coli* O157:H7. To address the concern of *E. coli* O157:H7, the industry has focused primarily on harvest interventions such as hot water, steam, and organic acid rinses to reduce pathogen contamination. Pathogen contamination is expected to be on the exterior carcass surfaces; however, it has been theorized that the fabrication process may reduce *E. coli* O157:H7 on subprimals – many of which are destined for the production of non-intact steaks and roasts. However, this has not been supported by peer-reviewed research. This study was conducted to determine the effectiveness of trimming original carcass surfaces during fabrication of subprimals on the reduction of *E. coli* O157:H7. The results support that trimming during the normal fabrication process may reduce surface contamination of *E. coli* O157:H7, but existing pathogens may spread to newly exposed surfaces. Intact muscles are considered sterile, so increased manipulation of whole muscles during fabrication may increase the contamination of external areas, whether original or newly exposed. This information will help the industry better understand how typical processing practices impact the safety of the products they produce.

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APPENDIX A

TABLE 1. *Least squares means for the daily initial inoculum concentration on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli

Day	log_{10} CFU/cm^2
1	5.1 A[a]
2	4.9 A

[a] Numbers with different letters significantly differ ($P < 0.05$).

TABLE 2. *Least squares means for initial inoculum location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli

Location [a]	log_{10} CFU/cm^2
Brisket area	5.1 A[b]
Plate area	5.0 A
Round area	5.0 A

[a] The location of samples taken were selected randomly along the length of the inoculated area of the carcass side to show the distribution of the inoculum.
[b] Numbers with different letters significantly differ ($P < 0.05$).

TABLE 3. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the brisket*

Location [a]	log_{10} CFU/cm^2
Fat, external, untrimmed	3.8 A[b]
Lean, initial	1.2 B
Fat, external, trimmed to 0.6 cm	1.8 B
Lean, trimmed	< 0.7 C[C]

[a] The initial fat samples were taken on the exterior carcass fat surface distal to the sternum. The trimmed fat samples were taken on the newly exposed fat surface. The initial lean samples were taken on the *M. pectoralis profundus* exposed after the sternum was removed. The lean trimmed samples were taken on the newly exposed *M. pectoralis profundus* surface after being trimmed flesh.
[b] Numbers with different letters significantly differ ($P < 0.05$).
[c] Value denotes samples below the minimum detection level of 0.7 log CFU/cm^2.

TABLE 4. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the chuck roll*

Location [a]	log_{10} CFU/cm^2
Fat, external, untrimmed	3.3 A[b]
Lean, initial	0.9 B
Lean trimmed	1.4 B

[a] The initial fat samples were taken on the exterior carcass fat surface near the dorsal anterior portion of the chuck primal. The initial lean samples were taken from the *M. longissimus thoracis* face where the rib was separated from the chuck (6th rib interface). The trimmed lean samples were taken on the dorsal aspect on the newly exposed lean surface where the external fat was removed.
[b] Numbers with different letters significantly differ ($P < 0.05$).

TABLE 5. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the clod*

Location [a]	log_{10} CFU/cm^2
Fat, external, untrimmed	3.7 A[b]
Lean, initial	1.1 B
Fat, external, trimmed to 0.6 cm	0.8 B

[a] The initial fat samples were taken on the external fat surface where the *M. cutaneous omobrachialis* was present. The trimmed fat samples were taken on the newly exposed fat surface. The initial lean samples were taken on the interior lean surface of the *M. infraspinatus* and *M. triceps brachii*.
[b] Numbers with different letters significantly differ ($P < 0.05$).

TABLE 6. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the rib*

Location [a]	log_{10} CFU/cm^2
Fat, external, untrimmed	2.9 A[b]
Lean, initial	< 0.7 C[C]
Fat, external, trimmed	0.9 BC
Lean, trimmed	1.1 B

[a] The initial fat samples were taken on the external fat surface over the *M. longissimus thoracis*. The trimmed fat samples were taken on the intermuscular fat surface on the dorsal aspect of the ribeye exposed after the lifter meat and all external fat was removed. The initial lean samples were taken on the *M. longissimus thoracis* face where the rib was separated from the chuck (6th rib interface). The trimmed lean sample was taken on the ventral lean surface exposed after the back ribs were removed.
[b] Numbers with different letters differ ($P < 0.05$).
[c] Value denotes samples below the minimum detection level of 0.7 log CFU/cm^2.

TABLE 7. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the bottom round*

Location [a]	log_{10} CFU/cm^2
Fat, external, untrimmed	2.6 A[b]
Lean, initial	1.1 B
Fat, external, trimmed to 0.6 cm	2.2 A
Lean, trimmed	0.8 B

[a] The initial fat samples were taken on the exterior carcass fat surface. The trimmed fat samples were taken on the newly exposed fat surface. The initial lean samples were taken on the ventral lean surface of the *M. semitendinosus*, *M. gluteobiceps*, and *M. gastrocnemius*. The trimmed samples were taken on the lean surface exposed after the heavy connective tissue (epimysium), *popliteal* lymph gland, *sarcosciatic* ligament, *M. gastrocnemius*, and *M. semitendinosus* were removed.
[b] Numbers with different letters significantly differ ($P < 0.05$).

TABLE 8. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the short loin*

Location [a]	log_{10} CFU/cm^2
Fat, external, untrimmed	2.3 A[b]
Lean, initial	0.9 B
Fat, external, trimmed to 0.6 cm	< 0.7 B[C]

[a] The initial fat samples were taken on the external fat surface that over the *M. longissimus lumborum*. The trimmed fat samples were taken on the newly exposed fat surface. The initial lean samples were taken from the *M. longissimus lumborum* face on the sirloin end where the short loin was separated from the sirloin (6th lumbar interface).
[b] Numbers with different letters significantly differ ($P < 0.05$).
[c] Value denotes samples below the minimum detection level of 0.7 log CFU/cm^2.

TABLE 9. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the top sirloin*

Location [a]	log_{10} CFU/cm^2
External fat	2.9 A[b]
Initial lean	1.7 B
Fat, external, trimmed to 0.6 cm	1.2 B

[a] The initial fat samples were taken on the exterior carcass fat surface. The trimmed fat samples were taken on the newly exposed fat surface. The initial lean samples were taken on the ventral lean surface exposed after the ilium and sacral bones were removed.

[b] Numbers with different letters significantly differ ($P < 0.05$).

TABLE 10. *Least squares means for fabrication location effect on counts (log_{10} CFU/cm^2) of rifampicin-resistant* E. coli *for the inside round*

Location [a]	log_{10} CFU/cm^2
Fat, external, untrimmed	3.5 A[b]
Lean, initial	0.9 C
Fat, external, trimmed to 0.6 cm	2.3 B
Lean, trimmed	< 0.7 C[C]

[a] The initial fat samples were taken on the exterior carcass fat surface. The trimmed fat samples were taken on the newly exposed fat surface. The initial lean samples were taken on the ventral lean surface of where the inside round was separated from the bottom round and knuckle. The trimmed lean samples were taken on the *M. semimembranosus* that was exposed and dried during chilling and trimmed away to expose a new surface.

[b] Numbers with different letters significantly differ ($P < 0.05$).

[c] Value denotes samples below the minimum detection level of 0.7 log CFU/cm^2.

APPENDIX B

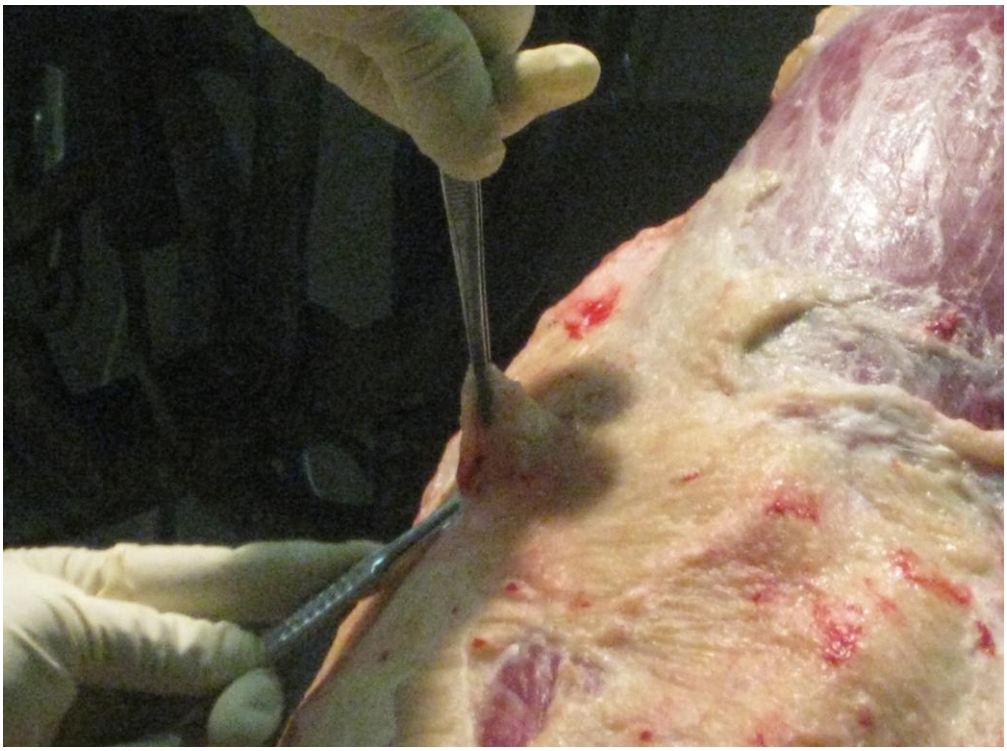

FIGURE 1. *Removing sample from the round area to measure background flora*.

FIGURE 2. *Typical pattern opening area where inoculation was applied (e.g., brisket, plate, flank, round, and leg extremities).*

VITA

Name:	Brittany Anise Laster
Education:	Texas A&M University B.S., Animal Science May 2009 Texas A&M University M.S., Animal Science (Meat Science) December 2010
Email Address:	brittany.a.laster@gmail.com
Address:	Texas A&M University Department of Animal Science College Station, TX 77843-2471